Example 30: Pharmacokinetic Studies

[1019] Assay was performed that detected antibody binding to ErbB2 receptor. (FIG. **13**) Assay was performed that detected at least two dolastatins linked to an antibody (FIG. **14**).

[1020] FIG. **15**.

Example 31: Treatment for Breast Cancer

[1021] Human Clinical Trial of the Safety and/or Efficacy of Trastuzumab-Linked Dolastatin Derivative for Breast Cancer Therapy

[1022] Objective: To compare the safety and pharmacokinetics of administered composition comprising trastuzumab-linked dolastatin derivative.

[1023] Study Design: This study will be a Phase I, single-center, open-label, randomized dose escalation study followed by a Phase II study in breast cancer patients. Patients should not have had exposure to trastuzumab-linked dolastatin derivative prior to the study entry. Patients must not have received treatment for their cancer within 2 weeks of beginning the trial. Treatments include the use of chemotherapy, hematopoietic growth factors, and biologic therapy such as monoclonal antibodies. Patients must have recovered from all toxicities (to grade 0 or 1) associated with previous treatment. All subjects are evaluated for safety and all blood collections for pharmacokinetic analysis are collected as scheduled. All studies are performed with institutional ethics committee approval and patient consent.

[1024] Phase I: Patients receive i.v. trastuzumab-linked dolastatin derivative on days 1, 8, and 15 of each 28-day cycle. Doses of trastuzumab-linked dolastatin derivative may be held or modified for toxicity based on assessments as outlined below. Treatment repeats every 28 days in the absence of unacceptable toxicity. Cohorts of 3-6 patients receive escalating doses of trastuzumab-linked dolastatin derivative until the maximum tolerated dose (MTD) for trastuzumab-linked dolastatin derivative is determined. The MTD is defined as the dose preceding that at which 2 of 3 or 2 of 6 patients experience dose-limiting toxicity. Dose limiting toxicities are determined according to the definitions and standards set by the National Cancer Institute (NCI) Common Terminology for Adverse Events (CTCAE) Version 3.0 (Aug. 9, 2006).

[1025] Phase II: Patients receive trastuzumab-linked dolastatin derivative as in phase I at the MTD determined in phase I. Treatment repeats every 4 weeks for 2-6 courses in the absence of disease progression or unacceptable toxicity. After completion of 2 courses of study therapy, patients who achieve a complete or partial response may receive an additional 4 courses. Patients who maintain stable disease for more than 2 months after completion of 6 courses of study therapy may receive an additional 6 courses at the time of disease progression, provided they meet original eligibility criteria.

[1026] Blood Sampling Serial blood is drawn by direct vein puncture before and after administration of trastuzumab-linked dolastatin derivative. Venous blood samples (5 mL) for determination of serum concentrations are obtained at about 10 minutes prior to dosing and at approximately the following times after dosing: days 1, 8, and 15, Each serum sample is divided into two aliquots. All serum samples are stored at -20° C. Serum samples are shipped on dry ice.

[1027] Pharmacokinetics: Patients undergo plasma/serum sample collection for pharmacokinetic evaluation before beginning treatment and at days 1, 8, and 15. Pharmacokinetic parameters are calculated by model independent methods on a Digital Equipment Corporation VAX 8600 computer system using the latest version of the BIOAVL software. The following pharmacokinetics parameters are determined: peak serum concentration (C_{max}); time to peak serum concentration (t_{max}); area under the concentration-time curve (AUC) from time zero to the last blood sampling time (AUC_{0-72}) calculated with the use of the linear trapezoidal rule; and terminal elimination half-life ($t_{1/2}$), computed from the elimination rate constant. The elimination rate constant is estimated by linear regression of consecutive data points in the terminal linear region of the log-linear concentration-time plot. The mean, standard deviation (SD), and coefficient of variation (CV) of the pharmacokinetic parameters are calculated for each treatment. The ratio of the parameter means (preserved formulation/non-preserved formulation) is calculated.

[1028] Patient Response to combination therapy: Patient response is assessed via imaging with X-ray, CT scans, and MRI, and imaging is performed prior to beginning the study and at the end of the first cycle, with additional imaging performed every four weeks or at the end of subsequent cycles. Imaging modalities are chosen based upon the cancer type and feasibility/availability, and the same imaging modality is utilized for similar cancer types as well as throughout each patient's study course. Response rates are determined using the RECIST criteria. (Therasse et al, J. Natl. Cancer Inst. Feb. 2, 2000; 92(3):205-16; http://ctep.cancer.gov/forms/TherasseRECISTJTNCI.pdf). Patients also undergo cancer/tumor biopsy to assess changes in progenitor cancer cell phenotype and clonogenic growth by flow cytometry, Western blotting, and IHC, and for changes in cytogenetics by FISH. After completion of study treatment, patients are followed periodically for 4 weeks.

Example 32: Treatment for Breast Cancer

[1029] Human Clinical Trial of the Safety and Efficacy of Trastuzumab-Linked Dolastatin Derivative for Breast Cancer Therapy

[1030] Objective: Compare the efficacy and toxicity of trastuzumab-linked dolastatin derivative alone followed at disease progression by combination trastuzumab and paclitaxel vs first-line combination trastuzumab and paclitaxel in women with HER2-overexpressing metastatic breast cancer.

[1031] Study Design: This study is a randomized, multicenter study. Patients are stratified according to degree of HER2/neu-overexpression (2+ vs 3+), prior anthracycline-containing adjuvant treatment (no prior treatment vs prior treatment without radiotherapy to left chest wall vs prior treatment with radiotherapy to left chest wall), estrogen-receptor status (positive vs negative vs unknown), prior therapy (first-line vs second/third-line), and center. Patients are randomized to one of two treatment arms. Arm I: Patients receive trastuzumab-linked dolastatin derivative IV over 30-90 minutes weekly. At time of disease progression, patients receive combination trastuzumab-linked dolastatin derivative IV and paclitaxel IV as in arm II. Arm II: Patients receive trastuzumab-linked dolastatin derivative IV over 30-90 minutes weekly. Paclitaxel is administered IV over 1 hour weekly for 3 weeks followed by 1 week of rest.